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Permalink

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Journal

Neuroscience, 84(1)

ISSN

0306-4522

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Publication Date

1998-05-01

DOI

10.1016/s0306-4522(97)00499-5

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Peer reviewed

Published in final edited form as:

Neuroscience. 1998 May ; 84(1): 71–79.

THE PRO-CONVULSANT ACTIONS OF CORTICOTROPIN-RELEASING HORMONE IN THE HIPPOCAMPUS OF INFANT RATS

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Abstract

Whole-cell patch-clamp and extracellular field recordings were obtained from 450- μ m-thick brain slices of infant rats (10–13 days postnatal) to determine the actions of corticotropin-releasing hormone on glutamate- and GABA-mediated synaptic transmission in the hippocampus. Synthetic corticotropin-releasing hormone (0.15 μ M) reversibly increased the excitability of hippocampal pyramidal cells, as determined by the increase in the amplitude of the CA1 population spikes evoked by stimulation of the Schaffer collateral pathway. This increase in population spike amplitude could be prevented by the corticotropin-releasing hormone receptor antagonist α -helical (9–41)-corticotropin-releasing hormone (10 μ M). Whole-cell patch-clamp recordings revealed that, in the presence of blockers of fast excitatory and inhibitory synaptic transmission, corticotropin-releasing hormone caused only a small (1–2 mV) depolarization of the resting membrane potential in CA3 pyramidal cells, and it did not significantly alter the input resistance. However, corticotropin-releasing hormone, in addition to decreasing the slow afterhyperpolarization, caused an increase in the number of action potentials per burst evoked by depolarizing current pulses. Corticotropin-releasing hormone did not significantly change the frequency, amplitude or kinetics of miniature excitatory postsynaptic currents. However, it increased the frequency of the spontaneous excitatory postsynaptic currents in CA3 pyramidal cells, without altering their amplitude and single exponential rise and decay time constants. Corticotropin-releasing hormone did not change the amplitude of the pharmacologically isolated (i.e. recorded in the presence of GABA_A receptor antagonist bicuculline) excitatory postsynaptic currents in CA3 and CA1 pyramidal cells evoked by stimulation of the mossy fibers and the Schaffer collaterals, respectively. Current-clamp recordings in bicuculline-containing medium showed that, in the presence of corticotropin-releasing hormone, mossy fiber stimulation leads to large, synchronized, polysynaptically-evoked bursts of action potentials in CA3 pyramidal cells. In addition, the peptide caused a small, reversible decrease in the amplitude of the pharmacologically isolated (i.e. recorded in the presence of glutamate receptor antagonists) evoked inhibitory postsynaptic currents in CA3 pyramidal cells, but it did not significantly alter the frequency, amplitude, rise and decay time constants of spontaneous or miniature inhibitory postsynaptic currents.

These data demonstrate that corticotropin-releasing hormone, an endogenous neuropeptide whose intracerebroventricular infusion results in seizure activity in immature rats, has diverse effects in the hippocampus which may contribute to epileptogenesis. It is proposed that the net effect of corticotropin-releasing hormone is a preferential amplification of those incoming excitatory signals which are strong enough to reach firing threshold in at least a subpopulation of CA3 cells.

These findings suggest that the actions of corticotropin-releasing hormone on neuronal excitability in the immature hippocampus may play a role in human developmental epilepsies.

Keywords

epilepsy; development; GABA; glutamate; excitability; seizure

The neuropeptide corticotropin-releasing hormone (CRH) may play an important role in seizure disorders of the developing brain. For example, massive infantile spasms, a particularly devastating and common form of pediatric epilepsy,^{1,14} respond to adrenocorticotrophic hormone, an agent which decreases the levels of CRH in the central nervous system.⁴ Recent evidence suggests that CRH may also play a central role in the generation of febrile seizures.⁹ Intracerebroventricular infusion of CRH results in seizures in young rats, whereas CRH-induced seizures are considerably less prominent in adult animals compared to infant rats.^{5,8,13,18} The CRH-induced seizures can be prevented by blocking CRH receptors.⁸ Receptors for CRH are found in brain areas known for their involvement in seizures, including the hippocampus, neocortex and amygdala.¹² In addition, CRH-containing cells and axons are also present in these brain regions.^{7,22,29} Of particular interest is the CA3 region of the hippocampus, where CRH receptor mRNA is highly abundant during early postnatal life,³ and CRH results in pyramidal cell damage in the CA3 subfield.⁶ The hippocampus is likely to play an important role in the generation of CRH-induced limbic seizures¹⁸ and/or in the propagation of seizures which originate elsewhere, e.g., in the amygdala.^{5,13}

In contrast to the considerable evidence implicating CRH in developmental seizures, our knowledge of the cellular–synaptic mechanisms involved in the generation of CRH-induced seizures is rather limited. Intracellular electrophysiological recordings carried out with sharp microelectrodes have shown that CRH decreases the slow afterhyperpolarization (AHP) following a burst of action potentials evoked by intracellular injection of depolarizing current in CA1 and CA3 pyramidal cells,^{2,23,24} as well as in the basolateral nucleus of the amygdala²¹ and in Purkinje cells of the cerebellum.¹⁵ In a recent study, Smith and Dudek²⁴ demonstrated that CRH (0.2 μ M) caused a 28% increase in the amplitude of the population spike in the CA1 region. This effect of CRH was larger in infant rats than in adults, and persisted in the presence of the GABA_A receptor antagonist bicuculline.²⁴ In the present study, we used whole-cell patch-clamp recording techniques *in vitro* to investigate the effects of CRH on synaptic transmission in the infant rat hippocampus.

EXPERIMENTAL PROCEDURES

Preparation of slices

The preparation of slices from infant rats was done as described previously for the adult.^{28,25} Briefly, 10- to 13-day-old Sprague–Dawley rats (Simonsen Laboratories Inc., Gilroy, CA, U.S.A.) were decapitated under halothane anesthesia and the brains were removed. Brains were cooled in 4°C artificial cerebrospinal fluid (ACSF), composed of (in mM): 126 NaCl, 2.5 KCl, 26 NaHCO₃, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄ and 10 glucose. Horizontal whole brain slices²⁷ (450 μ m) were prepared with the use of a Vibratome tissue sectioner (Lancer Series 1000). The brain slices were sagittally bisected into two hemispheric components and preincubated submerged in a temporary storage chamber containing oxygenated ACSF at 32°C for 1 h before any experimental manipulations.

Electrophysiology

Electrodes and solutions—Patch pipettes were pulled from borosilicate (KG-33) glass capillary tubing (1.5 mm o.d.; Garner Glass) with a Narishige PP-83 two-stage electrode puller. Tip dimensions were 0.5–1.0 μm inner diameter and 3–4 μm outer diameter. The intracellular solutions were made in high-performance liquid chromatography grade water (Omnisolve, EM Science). To record excitatory or inhibitory postsynaptic currents (EPSCs or IPSCs respectively), the intracellular solution consisted of (in mM): 140 cesium gluconate or CsCl, 2 MgCl_2 , 10 HEPES, 2 ATP (Sigma) and 11 EGTA (Sigma), and for spontaneous inhibitory currents, 3 mM QX-314; the pH was adjusted with CsOH to a final value of 7.20–7.25. The osmolarity of the intracellular solutions ranged between 255 and 270 mOsm. The solutions were filtered through a 0.2- μm pore size filter (Nalgene) before filling the pipettes. For extracellular field recordings, patch electrodes were filled with ACSF. All salts were obtained from Fluka.

Recordings—For electrophysiological recordings, the slices were transferred to the recording chamber perfused with ACSF containing the appropriate drugs for each experiment (as described below). Slices rested on filter paper and were stabilized with platinum wire weights. Slices were continuously superfused with warm, humidified 95% O_2 +5% CO_2 , and the temperature of the perfusion solution was maintained at 36°C. The application of the “blind” whole-cell recording technique to the standard brain slice preparation has been described in detail.^{10,28} Recordings were obtained with an Axopatch 200A amplifier (Axon Instruments), or with a Neurodata two-channel intracellular amplifier (Cygnus Technology), and digitized at 88 kHz before being stored on videotape.

To elicit orthodromic field potential responses in the CA1 region of the hippocampus, current pulses were delivered through a bipolar stimulating electrode placed in the stratum radiatum of CA1. CRH (Bachem, Torrance, CA, U.S.A.) was included in the perfusing solution and was bath applied. Whole-cell recordings of spontaneous, miniature and evoked synaptic currents were obtained from CA3 (and in some cases CA1) pyramidal cells in slices perfused with the appropriate solution with or without CRH: (i) for field recordings and spontaneous EPSCs (sEPSCs): control ACSF; (ii) for evoked EPSCs (eEPSCs): ACSF and 20 μM bicuculline methiodide (BMI; Research Biochem. Int.); (iii) for miniature EPSCs (mEPSCs): ACSF, BMI and 1 μM tetrodotoxin (TTX; Calbiochem); (iv) for spontaneous IPSCs (sIPSCs) and evoked IPSCs (eIPSCs): ACSF and 10 μM 2-amino-5-phosphovaleric acid (APV; Tocris)+5 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris); (v) for miniature IPSCs (mIPSCs): ACSF containing APV, CNQX and TTX. During the recordings, the series resistance (R_s) was closely monitored, and data were rejected if the R_s changed significantly or if it exceeded 16 M Ω .

Analysis—The recordings were filtered at 3 kHz before digitization at 20 kHz by computer (National Instruments LabPC+A/D board in a Pentium 90 MHz personal computer). To digitize and analyse the data, Strathclyde Electrophysiology software (courtesy of Dr J. Dempster) and Synapse software (courtesy of Dr Y. De Koninck) were used. Detection of individual spontaneous and miniature synaptic events was achieved using a software trigger described previously.^{20,26} For each experiment, all detected events were examined; any noise that spuriously met trigger specifications was rejected. A least-squares Simplex-based algorithm was used to fit the ensemble average with the sum of two (one rising and one decaying) exponentials:

$$I(t) = -A \cdot \exp^{-\tau_r/t} + A \cdot \exp^{-\tau_d/t},$$

where $I(t)$ is the synaptic current as a function of time (t), A is a constant, and τ_r and τ_D are the rise and decay time constants, respectively. Statistical analyses (t -test or F -test) were performed using SPSS for Windows or Sigma Plot, with a level of significance of P 0.05. Data are presented as means \pm S.E. (n is number of cells).

RESULTS

Corticotropin-releasing hormone reversibly increases the evoked population discharges of hippocampal principal cells

First, experiments were conducted to determine whether CRH increased the population responses in the CA1 region of immature rats. As Fig. 1 demonstrates, CRH (0.15 μ M) reversibly increased the population spike in CA1 following the first (asterisk in Fig. 1), as well as the second, stimulus of paired stimuli delivered to the Schaffer collateral pathway [Fig. 1; the paired-pulse protocol was employed to improve the ability to measure the increase in the population spike amplitude, since the population spike is usually small following the first stimulus in our slices in control medium (i.e. without BMI), because of the prominent feed-forward inhibition; for a detailed discussion, see Ref. 17. The increase in the population spike (123.6 \pm 5.9% of control; $n=3$) was significant ($P<0.05$), and comparable to that reported by Smith and Dudek.²⁴ Although CRH increased the population spikes, multiple discharges were not observed.²⁴

In a separate series of experiments, the effect of the CRH receptor antagonist α -helical (9–41)-CRH (10 μ M) was tested on the CRH-induced enhancement of the population spikes. CRH failed to increase the amplitude of the CA1 population spikes in the presence of CRH receptor antagonist ($n=3$), indicating that CRH increases neuronal excitability via CRH receptors.

Corticotropin-releasing hormone increases the intra-cellularly evoked burst firing in CA3 pyramidal cells, but it does not induce action potential firing when cells are at rest

Next, whole-cell patch-clamp experiments were conducted in CA3 pyramidal cells in current-clamp mode. The field recording experiments described above were conducted in CA1, to compare our data with those of Smith and Dudek,²⁴ and also because in infant rats the field potentials are considerably more robust and reliable in CA1 than in CA3. However, recent *in situ* hybridization data clearly showed that, within the hippocampus of infant rats, it is the CA3 region which contains the highest density of CRH receptor mRNA.³ Because of these data, in addition to the sensitivity of CA3 cells to CRH-induced seizures,⁶ and the known role of the CA3 region in seizure generation,³⁰ most of the whole-cell patch-clamp data reported in this paper were obtained from CA3 pyramidal cells.

In the presence of BMI, APV, and CNQX, CRH caused only a small (1–2 mV) positive shift in the resting membrane potential (V_m) of the whole-cell recorded CA3 pyramidal cells (pipette solution: potassium gluconate; $n=5$; cells were switched from control to CRH; control V_m before CRH application: -62.2 ± 1.83 mV; V_m in the presence of CRH: -61.2 ± 2.01 mV; note that although the means of the five pre-CRH V_m values and the means of the post-CRH V_m values appear to be similar, a paired t -test showed that the difference was significant; $P<0.05$). However, the small CRH-induced depolarization did not reach firing threshold in any of the cells. Therefore, CRH is unlikely to induce hyper-excitability *in vivo*⁸ by depolarizing the V_m and increasing the baseline spontaneous firing rate of CA3 cells.

However, in agreement with Aldenhoff *et al.*,² CRH caused a significant increase in the number of spikes in a burst evoked by a depolarizing current pulse ($n=3$ cells; the number of action potentials during the first 250 ms during the depolarizing current pulse in control

ACSF: 2.93 ± 0.13 ; in the presence of CRH: 4.36 ± 0.23), indicating that if a strong depolarizing influence and action potential firing are already present in the network, CRH can enhance excitability by increasing the number of action potentials that the cell emits in response to a given depolarizing input. Furthermore, as shown before,^{2,23,24} CRH decreased the slow AHP in the same cells. However, CRH did not change the input resistance (R_{IN} ; measured using hyperpolarizing responses from -75 mV in voltage clamp) of the CA3 pyramidal cells ($n=7$; control R_{IN} : 173.53 ± 26.3 M Ω R_{IN} in CRH: 154.18 ± 12.56 M Ω). Therefore, CRH does not in itself cause cells to fire, but if the neurons are brought to firing threshold by a depolarizing influence [e.g., an intracellular depolarizing current pulse or an excitatory postsynaptic potential (EPSP); see below], CRH can increase the number of action potentials and contribute to the spread of excitability in the network.

Effect of corticotropin-releasing hormone on spontaneous excitatory synaptic transmission in CA3 pyramidal cells

Next, sEPSCs were recorded with cesium gluconate-filled patch pipettes in control ACSF in CA3 pyramidal cells held at -60 mV (i.e. close to the reversal potential for sIPSCs, which we determined in each cell by establishing the membrane potential at which the high-frequency outward events, i.e. the sIPSCs, disappeared as the membrane potential was hyperpolarized from 0 mV). Under these conditions, sEPSCs appear as transient inward currents. Application of CRH significantly increased the frequency of the sEPSCs in six of seven cells, by $252.82 \pm 65.09\%$ on average (Fig. 2; average control sEPSC frequency in the six cells in which CRH did increase the sEPSC frequency: 1.95 ± 0.69 Hz; in the presence of CRH: 5.07 ± 2.02 ; average control sEPSC frequency in all seven cells: 2.57 ± 0.85 Hz; in the presence of CRH: 5.18 ± 1.71 Hz). The increase in frequency was fully reversible after switching back to control solution (Fig. 2C). The CRH-induced change in frequency was not accompanied by a change in sEPSC amplitude (control: 39.05 ± 3.92 pA; CRH: 43.32 ± 4.67 pA; $n=6$) or kinetics (rise time constants: control, 1.08 ± 0.16 ms; CRH, 1.38 ± 0.16 ms; decay time constants: control, 6.69 ± 0.75 ms; CRH, 7.02 ± 0.84 ms; $n=6$).

Corticotropin-releasing hormone has no effect on the frequency, amplitude and kinetics of miniature excitatory postsynaptic currents

The next series of experiments were aimed at determining whether CRH increases excitatory neurotransmission in the absence of action potentials. To achieve this goal, mEPSCs were recorded in the presence of the sodium channel blocker TTX, and BMI, with cesium gluconate electrodes, at -60 mV. No significant increase in the frequency of mEPSCs occurred when the control medium was switched to a medium containing CRH (control: 3.9 ± 3.1 Hz; CRH: 5.3 ± 4.3 ; $n=5$). When mEPSCs were compared in CA3 cells from slices bathed in either control ACSF or in CRH, the frequency of mEPSCs was also not significantly different (control: 2.2 ± 1.4 , $n=11$; CRH: 3.7 ± 2.2 , $n=10$). CRH did not alter the amplitude (control: 23.5 ± 1.6 pA, $n=5$; CRH: 21.9 ± 1.7 pA; $n=8$), rise (control: 1.0 ± 0.14 ms, $n=5$; CRH: 0.99 ± 0.13 ms, $n=8$) or decay time constants (control: 7.2 ± 0.86 ms, $n=5$; CRH: 6.8 ± 0.67 ms, $n=8$) of mEPSCs, indicating the lack of a direct postsynaptic action of CRH on glutamate receptors.

Corticotropin-releasing hormone does not alter the amplitude of evoked monosynaptic excitatory post-synaptic currents, but it can enhance the polysynaptic excitatory response

In the next series of experiments, the effect of CRH on the eEPSCs was determined. In the presence of BMI, CRH failed to alter the amplitude of the eEPSCs (Fig. 3A) evoked by stimulation of the mossy fiber pathway in CA3 cells ($n=7$; the amplitude of the eEPSCs was $106.87 \pm 16.01\%$ of control). Similarly, CRH did not change the amplitude of the eEPSCs in CA1 pyramidal cells evoked by stimulation of the Schaffer collaterals, in slices in which the CA1 and CA3 regions were separated by a knife-cut in order to eliminate the spread of the

BMI-induced epileptiform activity from CA3 to CA1 ($n=4$; Fig. 3A). These results are in agreement with the lack of CRH effects on the amplitude of the sEPSCs, and they indicate that CRH does not alter the action potential-induced release of glutamate.

Taken together, the data presented above suggest that CRH does not cause large depolarizations and induce action potential firing, does not alter post-synaptic glutamate receptors, and has no effect on the action potential-induced glutamate release, but the peptide can enhance the number of action potentials in a burst evoked by intracellular injection of depolarizing current pulses and increase the frequency of sEPSCs. Based on these data, it seems reasonable to suggest that although CRH by itself (i.e. when cells are at rest) cannot cause hyperexcitability (e.g., synchronized burst firing), it may enhance excitability when a depolarizing input is provided to bring at least some cells to firing threshold. Therefore, we tested this hypothesis by recording from CA3 pyramidal cells in the presence of BMI in current-clamp mode, and stimulated the mossy fiber pathway. If the hypothesis is correct, CRH should not alter the short-latency, monosynaptic EPSPs; however, provided that the stimulus can evoke firing, at least in some CA3 cells (perhaps occasionally also aided by the CRH-induced increase in the frequency of the randomly occurring sEPSCs), CRH may lead to burst firing at longer latencies in CA3 cells following stimulation of the mossy fiber pathway. Indeed, as shown in Fig. 3B, when the mossy fiber-evoked responses were recorded from CA3 pyramidal cells in current-clamp mode in the presence of BMI, CRH caused the appearance of large bursts of action potentials at variable latency, even though the amplitude of the monosynaptic EPSP did not change. The bursts of action potentials appeared to be elicited by longer-latency and most likely polysynaptic EPSPs. Upon switching back to the control solution, recovery could be obtained (in fact, in the cell shown in Fig. 3B, the effect of CRH could be repeated twice with full recovery). Therefore, similar to the ability of CRH to increase the number of action potentials in an intracellularly evoked burst, CRH can cause hyperexcitability in the network, provided that some excitatory influence (in this case provided by stimulation of the mossy fibers) is already present. In other words, CRH appears to amplify excitatory inputs, but it cannot cause hyperexcitability without them.

Corticotropin-releasing hormone's actions on inhibitory synaptic currents in CA3 cells

Next, we tested the effects of CRH on inhibitory synaptic transmission. In contrast to its action on sEPSCs, CRH did not significantly alter the frequency of sIPSCs, recorded in the presence of the glutamate receptor antagonists APV+CNQX, with CsCl-filled pipettes at -60 mV (sIPSC frequency in CA3 cells from slices incubated in control ACSF: 44.7 ± 4.2 Hz, $n=12$; in cells incubated in CRH-containing medium: 34.5 ± 5.0 Hz, $n=13$). Similarly, CRH did not alter the frequency of sIPSCs in cells where the perfusate was switched from control ACSF+APV+CNQX to ACSF+APV+CNQX+ CRH (Fig. 4A, B; $n=2$). Next, the effect of CRH on mIPSCs was tested, in ACSF containing APV+CNQX+TTX, at -60 mV with CsCl-filled pipettes. In three cells where the perfusate was switched from ACSF containing APV+CNQX+TTX to the same solution with added CRH, no significant alteration was found in the frequency (control: 1.3 ± 0.7 Hz; CRH: 0.7 ± 0.2 Hz), rise time constant (control: 0.8 ± 0.1 ms; CRH: 0.7 ± 0.1 ms), decay time constant (control: 7.5 ± 0.6 ms; CRH: 8.5 ± 0.5 ms) or amplitude (control: 71.0 ± 1.5 pA; CRH: 75.5 ± 8.1 pA) of mIPSCs in CA3 pyramidal cells. However, as shown in Fig. 4C, CRH was able to cause a small but significant and reversible decrease in the amplitude of eIPSCs evoked in the presence of APV and CNQX by a stimulating electrode placed in the CA3 pyramidal cell layer (control eIPSC amplitude: 2.16 ± 0.69 nA; eIPSC amplitude in the presence of CRH: 1.59 ± 0.56 nA; a 28.6% decrease, $n=5$; $P < 0.05$, paired t -test). These data indicate that CRH may induce a relatively small decrease in the action potential-induced release of GABA, but it has no

effect on the postsynaptic GABA_A receptors, or on the properties of the action potential-independent GABA release.

DISCUSSION

The main findings of this paper are that: (i) CRH causes a small depolarization of the membrane potential in CA3 cells, but the CRH-induced depolarization is not sufficient to bring cells to firing threshold; (ii) CRH, in addition to its known effect on the AHP, increases the number of spikes evoked by intracellular depolarizing current pulses; (iii) CRH increases the frequency of sEPSCs; (iv) CRH enhances polysynaptic burst firing in the network in response to mossy fiber stimulation; and (v) CRH causes a small decrease in the action potential-dependent GABA release. However, (vi) CRH has no direct postsynaptic actions on glutamate or GABA_A receptors mediating fast synaptic transmission, (vii) CRH does not alter the amplitude of the evoked, monosynaptic EPSCs, and (viii) CRH does not alter the action potential-independent glutamate and GABA release. These data suggest that CRH, by amplifying excitatory inputs reaching the CA3 network, can induce pro-convulsant effects within the CRH receptor-rich CA3 area, which may play an important role in the propagation of seizures in the developing brain.

Corticotropin-releasing hormone-induced hyperexcitability in the hippocampus

It has been well documented that CRH administered to developing animals induces seizures.^{5,8} Where these seizures originate is not clear at present. The electroencephalographic studies of Marrosu *et al.*¹⁸ suggested that the primary site of seizure-like electrical activity may be the hippocampus. However, recent evidence indicates that CRH-induced seizures originate from the amygdala, and the seizures then spread to the hippocampus.^{5,13} Our field recording results, in agreement with previous *in vitro* data,²⁴ suggest that CRH alone does not lead to obvious seizure-like electrical population activity in the hippocampus. On the other hand, the pro-convulsive actions of CRH in the hippocampus reported in this paper are likely to augment seizure activity, originating either in the hippocampus or elsewhere (e.g., in the amygdala), and subsequently spreading to the hippocampal formation.

Cellular–synaptic mechanisms underlying corticotropin-releasing hormone-induced hyperexcitability

Although it is clear that CRH induces seizures in infant animals, the mechanisms underlying this phenomenon are not well understood. Previous investigations, all carried out with sharp microelectrodes, determined that CRH reduces the AHP, resulting in an increased number of spikes and increased burst duration in response to a pulse of depolarizing current.^{2,15,21,23,24} However, whether this effect of CRH on the AHP leads to increased excitatory synaptic communication has not been studied in detail. Aldenhoff *et al.*² noted that the frequency of sub-threshold activity increased after CRH application, and Smith and Dudek²⁴ found variable effects of CRH on synaptic transmission in their study carried out with sharp electrodes in CA1 pyramidal cells. We have employed high-resolution patch-clamp techniques in CA3 pyramidal cells to study the synaptic effects of CRH in infant rats *in vitro*.

Our data showed that CRH has a variety of potentially excitability-increasing effects on single CA3 pyramidal cells in the hippocampus, including a small depolarization, a decrease in AHP and an increase in evoked burst firing. These actions of CRH on the intrinsic properties of CA3 cells do not, by themselves, lead to overt hyperexcitability (e.g., burst firing) in the network. Similarly, CRH does not cause an increase in the monosynaptically evoked EPSCs in either CA3 or CA1 cells. However, CRH is able to greatly amplify mossy

fiber stimulation-evoked poly-synaptic excitability in the CA3 network (e.g., Fig. 3B), and it can also increase the frequency of sEPSCs. Therefore, when some level of excitability is present (either as a result of activation of excitatory inputs or spontaneous discharges of pyramidal cells), CRH can lead to hyperexcitability, most likely via the increase in the number of spikes that CA3 cells emit either in response to a depolarizing input or spontaneously.

Although CRH can increase excitability even in the presence of BMI, as shown by Smith and Dudek²⁴ and by our data, CRH also has a clear, albeit small, effect on action potential-mediated GABA release. Interestingly, although CRH decreases the monosynaptically evoked GABA_A response, it did not cause a significant decrease in the frequency of sIPSCs (although there did appear to be a small, non-significant decrease, e.g., Fig. 4A, B). The most plausible explanation for this seems to be that CRH may cause an increase in firing of some interneurons, which may counteract the small decrease in the action potential-dependent GABA release. Direct recording from various interneuronal populations¹⁹ will be necessary to test this hypothesis. The possibility that some interneuronal classes may be influenced by CRH is especially interesting in light of the observation that CRH may be present exclusively in non-principal cells in the CA3 and CA1 fields,⁷ some of which may innervate other interneuronal populations (for reviews, see Refs 11 and 16).

These data indicate that CRH has various pro-convulsant actions in the hippocampus, and thus support the suggested role of CRH in certain non-genetic seizures of the developing human.^{4,8} Endogenous CRH functions as a stress neurohormone, and a pathological increase in CRH release is likely under circumstances of stress or insult to the developing central nervous system. Such circumstances are associated with febrile seizures and infantile spasms, seizures which are restricted to infancy and early childhood in human. Infantile spasms, in particular, are eliminated by agents (such as adrenocorticotrophic hormone) which down-regulate CRH secretion.⁴ The current study demonstrates that CRH, especially in the presence of depolarizing inputs, leads to increased excitability in the CA3 network. The CRH-induced increase in action potential burst firing would enhance glutamate release in a vicious circle that may contribute to epileptogenesis and/or to the degeneration of limbic neurons, as has been observed after CRH administration in the developing rat.⁶

CONCLUSIONS

The data presented in this paper demonstrate that CRH has diverse pro-convulsant effects in the hippocampus, and it is proposed that CRH amplifies incoming excitatory signals and thus contributes to hyperexcitability. Future research will be necessary to determine the precise molecular mechanisms involved in the various effects of CRH, the age dependence of its actions,²⁴ and the cellular identity and connectivity of CRH-containing neurons⁷ in epilepsy-prone brain areas such as the hippocampus and the amygdala.

Acknowledgments

We thank Ms G. Sandor and Ms M. He for expert technical assistance, Dr J. Dempster for providing the Strathclyde Electrophysiology Software, and Dr Y. De Koninck for the Synapse software. The work was financially supported by March of Dimes Basil O'Connor Research Scholar Award (5-FY95-1143) and by the American Epilepsy Society (EFA-21311) to I. Soltesz, and NIH NS28912 to T. Z. Baram.

Abbreviations

| | |
|------|--------------------------------|
| ACSF | artificial cerebrospinal fluid |
| AHP | afterhyperpolarization |

| | |
|--------------|--|
| APV | 2-amino-5-phosphovaleric acid |
| BMI | bicuculline methiodide |
| CNQX | 6-cyano-7-nitroquinoxaline-2,3-dione |
| CRH | corticotropin-releasing hormone |
| eEPSC | evoked excitatory postsynaptic current |
| EGTA | ethyleneglycolbis(aminoethyl ether) tetra-acetate |
| eIPSC | evoked inhibitory postsynaptic current |
| EPSC | excitatory postsynaptic current |
| EPSP | excitatory postsynaptic potential |
| HEPES | <i>N</i> -2-hydroxyethylpiperazine- <i>N'</i> -2-ethanesulfonic acid |
| IPSC | inhibitory postsynaptic current |
| mEPSC | miniature excitatory postsynaptic current |
| mIPSC | miniature inhibitory postsynaptic current |
| sEPSC | spontaneous excitatory postsynaptic current |
| sIPSC | spontaneous inhibitory postsynaptic current |
| TTX | tetrodotoxin |

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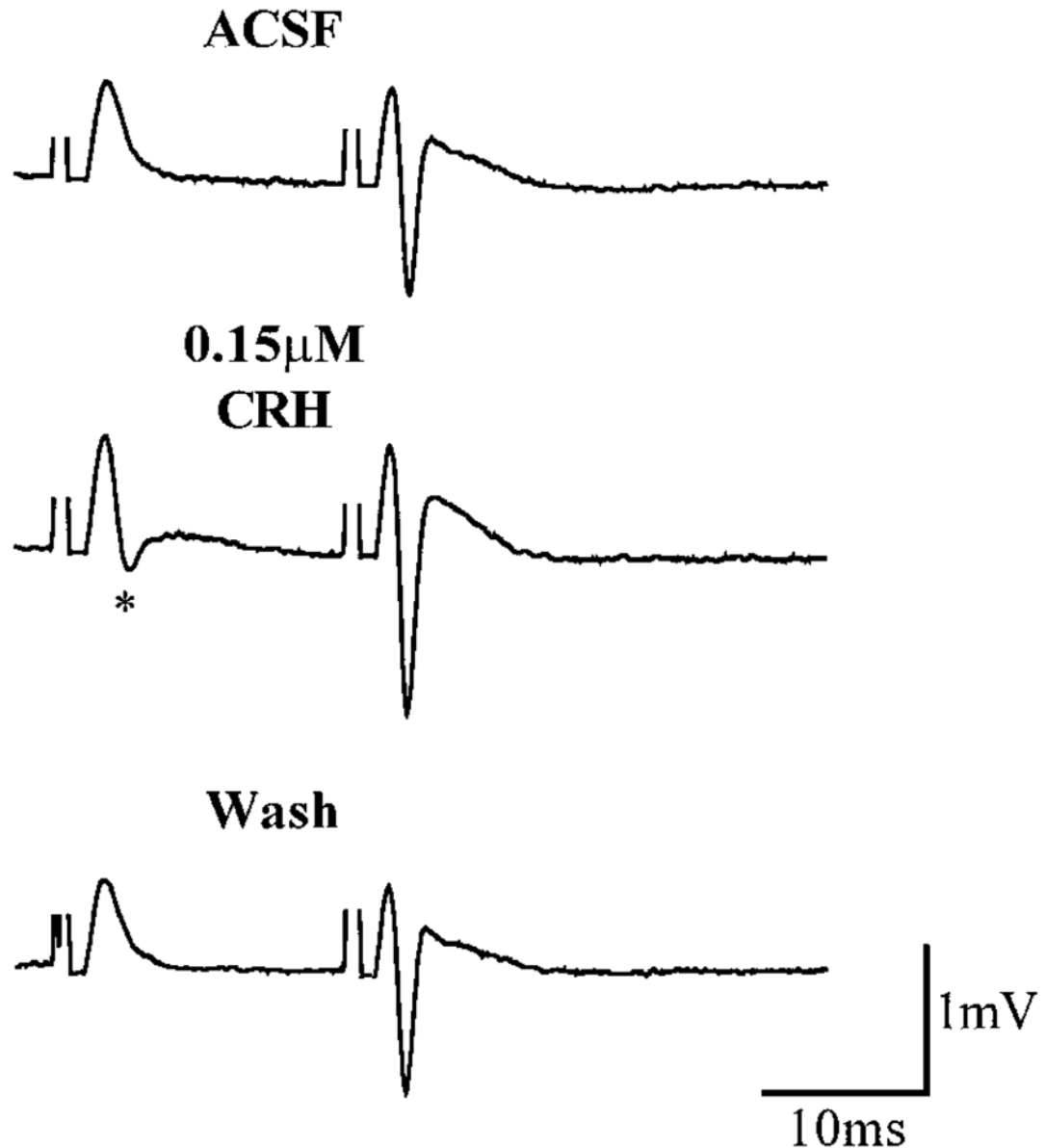
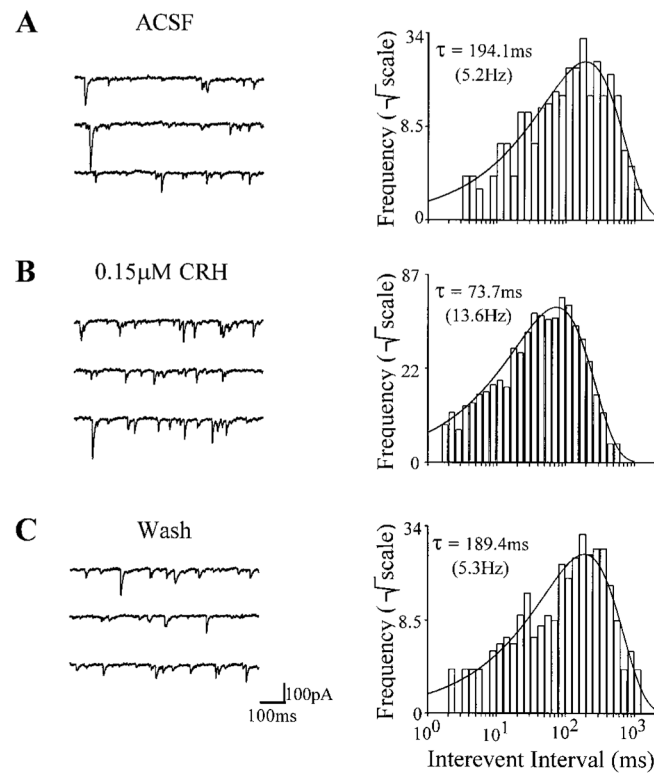
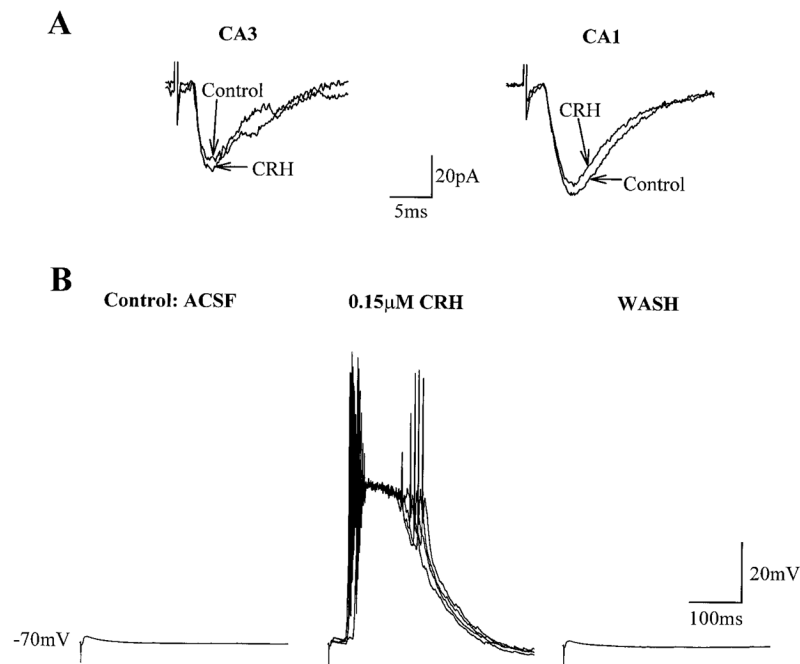


Fig. 1.

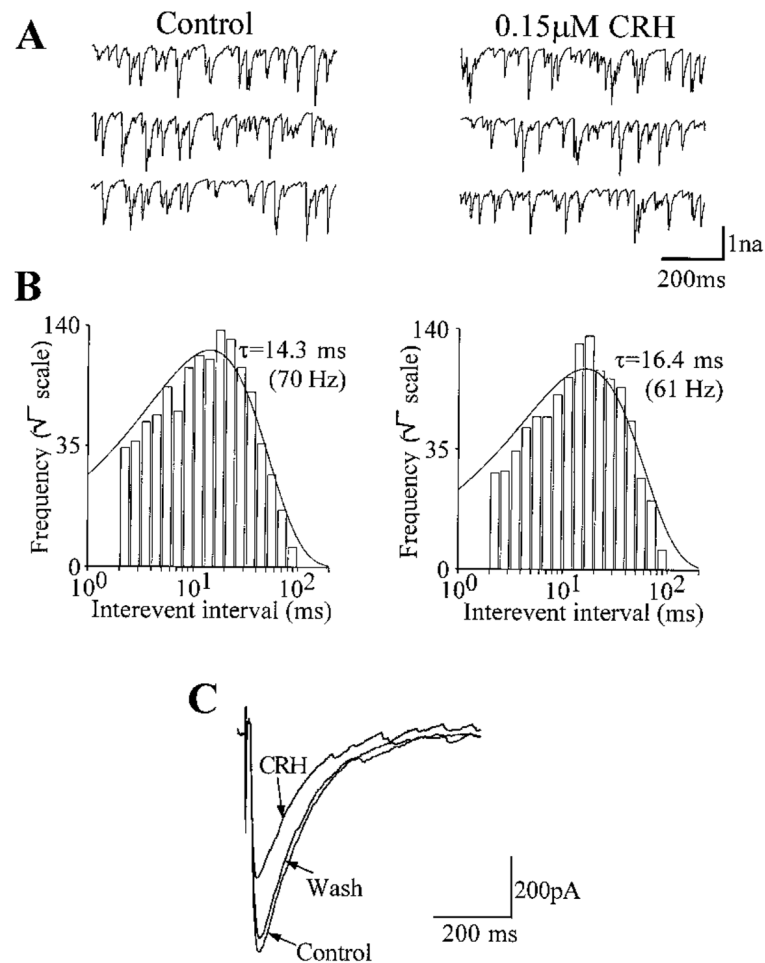
CRH reversibly increases the evoked population spikes. Field recording in the CA1 pyramidal cell layer shows the response to a paired-pulse stimulus delivered to the Schaffer collateral pathway in the stratum radiatum. Note that CRH (0.15 μ M) caused the appearance of a population spike (asterisk) following the first stimulus, and also increased the amplitude of the population spike after the second stimulus. After washout of the drug, full recovery could be obtained. Both the CRH effect and the washout occurred relatively rapidly, in 5–6 and 10–15 min, respectively (in our system BMI abolishes mIPSCs in 3 min).

**Fig. 2.**

CRH reversibly increases the frequency of sEPSCs in CA3 pyramidal cells. (A) Whole-cell recording in control ACSF, with cesium gluconate-filled electrodes at -60 mV (close to the reversal potential of IPSCs), shows spontaneous EPSCs occurring at 5.2 Hz (as shown by the inter-event interval distribution on the right) from a CA3 pyramidal cell. (B) Seven minutes after switching to ACSF containing CRH ($0.15\text{ }\mu\text{M}$), the frequency of the sEPSCs increased to 13.6 Hz. CRH caused a similar increase (253% on average) in sEPSC frequency in six of seven cells. (C) The effect of CRH on sEPSC frequency was fully reversible.

**Fig. 3.**

The effect of CRH on evoked excitatory responses in the presence of bicuculline. (A) Voltage-clamp recording shows that CRH does not change the monosynaptically evoked EPSCs in CA3 or CA1 cells following stimulation of the mossy fiber and the Schaffer collateral pathway, respectively. (B) When the experiment was repeated in current-clamp mode, CRH lead to large, synchronized polysynaptic discharges in CA3 cells following mossy fiber stimulation. Note that the bursts have variable and long latency (indicating the polysynaptic nature of the response), and they appear to rise from EPSPs, and that the monosynaptic EPSP did not change in amplitude.

**Fig. 4.**

CRH does not significantly alter the frequency of sIPSCs, but it causes a small decrease in the amplitude of eIPSCs. (A) Examples of sIPSCs are shown, recorded in the presence of APV+CNQX, at -60 mV with CsCl-filled pipettes from a CA3 pyramidal cell. As illustrated by the inter-event interval distribution below, the frequency of sIPSCs was 70 Hz. (B) Nine minutes after switching to a solution containing CRH ($0.15 \mu\text{M}$), there was no significant change in the frequency of sIPSCs (61 Hz). (C) In CA3 pyramidal cells, CRH caused a reversible, small (28.6%) decrease in the amplitude of IPSCs evoked by a stimulating electrode placed in the pyramidal cell layer, in the presence of APV and CNQX.